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p120 and Kaiso Regulate *Helicobacter pylori*-induced Expression of Matrix Metalloproteinase-7

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***Helicobacter pylori* is the strongest known risk factor for gastric adenocarcinoma, yet only a fraction of infected persons develop cancer. One *H. pylori* constituent that augments disease risk is the cytotoxin-associated gene (*cag*) pathogenicity island, which encodes a secretion system that translocates bacterial effector molecules into host cells. Matrix metalloproteinase (MMP)-7, a member of a family of enzymes with tumor-initiating properties, is overexpressed in premalignant and malignant gastric lesions, and *H. pylori cag*$^+$ strains selectively increase MMP-7 protein levels in gastric epithelial cells in vitro and in vivo. We now report that *H. pylori*-mediated *mmp-7* induction is transcriptionally regulated via aberrant activation of p120-catenin (p120), a component of adherens junctions. *H. pylori* increases *mmp-7* mRNA levels in a *cag*- and p120-dependent manner and induces translocation of p120 to the nucleus in vitro and in a novel ex vivo gastric gland culture system. Nuclear translocation of p120 in response to *H. pylori* relieves Kaiso-mediated transcriptional repression of *mmp-7*, which is implicated in tumorigenesis. These results indicate that selective and coordinated induction of *mmp-7* expression by *H. pylori cag*$^+$ isolates may explain in part the augmentation in gastric cancer risk associated with these strains.**

INTRODUCTION

Helicobacter pylori induces an inflammatory response in the stomach that persists for decades, and biological costs incurred by this pathogen include an increased risk for gastric adenocarcinoma and non-Hodgkins lymphoma of the stomach (Nomura *et al*., 1991; Parsonnet *et al*., 1991; Peterson, 1991; Hansson *et al*., 1993; Correa, 1996; Uemura *et al*., 2001; Peek and Blaser, 2002; Moss and Sood, 2003). However, only a fraction of colonized persons ever develop neoplasia, and enhanced cancer risk is related to strain-specific differences, aberrant host responses, and/or specific interactions between microbial and host determinants.

H. pylori strains that possess the cytotoxin-associated gene (*cag*) pathogenicity island increase the risk for cancer compared with strains that lack this genetic locus (Peek and Blaser, 2002). The *cag* island encodes proteins, such as CagE, that form a type IV secretion system that translocates components of bacterial peptidoglycan and CagA, the product of the terminal gene of the island, into host cells (Asahi *et al*., 2000; Backert *et al*., 2000; Odenbreit *et al*., 2000; Stein *et al*., 2000; Selbach *et al*., 2002; Viala *et al*., 2004). After translocation, peptidoglycan initiates innate immune signaling via activation of the intracellular pattern recognition receptor, Nod-1, and the transcriptional activator nuclear factor-κB (NF-κB) (Viala *et al*., 2004). Intracellular CagA undergoes Src-dependent tyrosine phosphorylation and activates a eukaryotic phosphatase, leading to dephosphorylation of host cell proteins and cellular morphological changes (Backert *et al*., 2000; Higashi *et al*., 2002; Selbach *et al*., 2002; Stein *et al*., 2002). Recently, CagA has been shown to activate β-catenin and induce NF-κB–mediated interleukin-8 release from gastric epithelial cells (Brandt *et al*., 2005; Franco *et al*., 2005). The presence of the *cag* island also influences the topography of colonization in the stomach, because *H. pylori cag*$^-$ strains predominate within the mucus gel layer, whereas *cag*$^+$ strains are found immediately adjacent to epithelial cells (Camorlinga-Ponce *et al*., 2004).

Matrix metalloproteinase (MMP)-7 is a member of a family of zinc-dependent proteolytic enzymes with tumor-initiating properties and is expressed and secreted by epithelial cells (Coussens *et al*., 2002; Egeblad and Werb, 2002). We and others have previously demonstrated that *H. pylori cag*$^+$ strains selectively up-regulate MMP-7 protein levels in gastric epithelial cells (Crawford *et al*., 2003; Wroblewski *et al*., 2003). Overexpression of MMP-7 occurs in premalignant and malignant gastric lesions (McDonnell *et al*., 1991; Honda *et al*., 1996; Saarialho-Kere *et al*., 1996; Adachi *et al*., 1998; Senota *et al*., 1998; Yamashita *et al*., 1998; Ajisaka *et al*., 2001; Hippo *et al*., 2002), and genetic polymorphisms linked to increased MMP-7 expression are associated with *H. pylori*

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Abbreviations used: *cag*, cytotoxin-associated gene; MMP-7, matrix metalloproteinase-7; p120, p120-catenin.

infection status, gastric ulceration (a precursor for gastric cancer), and tumor-related survival among gastric cancer patients (Hellmig *et al*., 2006; Kubben *et al*., 2006). In mice, overexpression of MMP-7 leads to hyperproliferation and increased cancer susceptibility (Rudolph-Owen *et al*., 1998), and cell lines that overexpress MMP-7 develop enhanced tumorigenic potential (Witty *et al*., 1994). Conversely, mice with a genetic predisposition for intestinal adenocarcinoma that are then bred onto a background of MMP-7 deficiency develop fewer cancers than wild-type mice (Wilson *et al*., 1997). Together, these data suggest that MMP-7 may play an important role early in gastric carcinogenesis.

A host molecule that has been implicated in regulation of MMP-7 expression is p120-catenin (p120). p120 was originally identified as a substrate for Src- and receptor-tyrosine kinases (Reynolds *et al*., 1989, 1992; Reynolds and Carnahan, 2004) and is a member of the catenin (ctn) family, an Armadillo domain protein subfamily whose members interact with the cadherin cytoplasmic tail and modulate cadherin function (Reynolds *et al*., 1994; Shibamoto *et al*., 1995; Staddon *et al*., 1995; Reynolds and Carnahan, 2004). Aberrant redistribution of p120 has been observed in several epithelial malignancies, including gastric cancer (Jawhari *et al*., 1999; Karatzas *et al*., 1999; Karayiannakis *et al*., 1999; Thoreson and Reynolds, 2002; Mayerle *et al*., 2003). Typically found at low levels in the nuclei of normal cells, increased levels of p120 have been observed in nuclei of tumor cells (Mayerle *et al*., 2003; Wijnhoven *et al*., 2005; Sarrio *et al*., 2006), and recent evidence has revealed that nuclear p120 acts to relieve transcriptional repression mediated by Kaiso, a member of the broad complex, tramtrak, bric a brac/pox virus, and zinc finger family (BTB/POZ) (Daniel and Reynolds, 1999). The Kaiso/p120 complex can modulate noncanonical Wnt signaling (Kim *et al*., 2004), and, along with T cell factor (TCF)/β-catenin complexes, coordinately regulate canonical Wnt gene targets such as *cyclin D1* and *mmp-7* (Park *et al*., 2005; Spring *et al*., 2005), both of which are up-regulated by *H. pylori* (Hirata *et al*., 2001; Bebb *et al*., 2003; Crawford *et al*., 2003; Wroblewski *et al*., 2003; Chang *et al*., 2006). Because MMP-7 exerts cancer-initiating properties and is specifically induced by contact with *H. pylori* in vitro and in vivo, we sought to define the molecular pathways underpinning increased MMP-7 expression to define a potential tumor-promoting response to this pathogen.

MATERIALS AND METHODS

H. pylori Strains

The *H. pylori* strains 7.13 or SS1 were grown in *Brucella* broth with 5% fetal bovine serum (FBS) for 18 h, with shaking in an atmosphere of 5% CO_2 at 37°C. Then, they were harvested by centrifugation and added to gastric epithelial cells at a multiplicity of infection (MOI) of 100. The 7.13 isogenic *cagA*$^-$ and *cagE*$^-$ null mutant strains were constructed by insertional mutagenesis using *aphA* (conferring kanamycin resistance) (Franco *et al*., 2005) and were selected on *Brucella* agar containing 25 μg/ml kanamycin. For luciferase assays, *H. pylori* strains were cultured on agar plates containing 10% horse serum in an atmosphere of 5% CO_2 at 37°C for 48 h. Bacteria were harvested in PBS, pH 7.4, added to host cells at an MOI of 100, and routinely monitored using an inverted microscope (model TS 100; Intas, Göttingen, Germany).

Cell Culture and Reagents

MKN28 human gastric epithelial cells (kindly provided by Dr. Robert Coffey, Vanderbilt University) were grown in RPMI 1640 medium (Mediatech, Herndon, VA) supplemented with 10% heat-inactivated FBS, 2 mM L-glutamine, and 20 μg/ml gentamicin in an atmosphere of 5% CO_2 at 37°C. Phoenix 293 cells (generously provided by Dr. Todd Graham, Vanderbilt University) were grown in DMEM supplemented with 10% heat-inactivated FBS, 2 mM L-glutamine, 50 U of penicillin/ml, and 50 μg of streptomycin/ml in an atmosphere of 5% CO_2 at 37°C.

Primary Gastric Cell Extraction and Culture

All animal studies were approved by the Vanderbilt Institutional Animal Care and Usage Committee. Stomachs were removed from killed 8-wk-old male FVB/n mice (Harlan, Indianapolis, IN), ligated at the pylorus and esophagus, inverted, and injected with 1 ml of 0.5 mg/ml collagenase A as described previously (Wroblewski *et al*., 2003). Stomachs were then washed in Hanks' balanced salt solution (HBSS) three times at 37°C. Tissue was incubated in 10 ml of 1 mM dithiothreitol for 15 min at 37°C with shaking, washed in HBSS three times at 37°C, and incubated in 0.37 mg/ml collagenase for 30 min at 37°C. After the first collagenase digestion, samples were washed again in HBSS (3 times at 37°C) and incubated for a further 30 min in collagenase (0.37 mg/ml; 37°C). Tissue was triturated using a wide-mouthed pipette, and larger fragments of tissue were allowed to settle under gravity for 45 s. The supernatant containing isolated gastric cell colonies was removed and transferred to a clean 50-ml conical tube, shaken vigorously to release additional cell colonies, and left on ice to sediment for 45 min. The supernatant was then carefully removed and discarded, and isolated cell colonies were plated in chamber slides. Colonies of gastric epithelial cells were cultured in DMEM NUT Mix F-12 (Ham's) supplemented with 10% FBS and 1% antibiotic-antimycotic solution. Then, colonies were incubated in a humidified incubator at 37°C under an atmosphere of 5% CO_2. Cell colonies were cultured for up to 72 h, and the medium was changed every 24 h.

Small Interfering RNA (siRNA) Constructs

pSUPER.retro.puro (Oligoengine, Seattle, WA) plasmid containing a human-specific targeting sequence directed toward p120 was kindly provided by Dr. Albert Reynolds (Vanderbilt University) (Davis *et al*., 2003; Mariner *et al*., 2004). pSUPER.retro.puro (Oligoengine) plasmid containing a scrambled nontargeting sequence was kindly provided by Dr. Howard Crawford (SUNY, Stony Brook). Nontargeting siRNA oligos (D-001210-01) or ON-TARGETplus SMARTpool siRNA oligonucleotides (oligos) directed toward Kaiso (ZBTB33; L-019982-00) were purchased from Dharmacon RNA Technologies (Lafayette, CO).

Viral Production and Retroviral Transduction

Phoenix 293 packaging cell lines at 50% confluence were transfected using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Fresh medium was added 24 h after transfection, and tissue culture medium was collected and filtered through a 0.45-μm filter 72 h after transfection. For retroviral transduction, MKN28 cells at 50% confluence were incubated overnight with freshly harvested virus containing 4 μg/ml Polybrene (American Bioanalytical, Georgetown, ON, Canada). To generate stable cell lines, cells transduced with the pSUPER.retro.puro virus were selected with 1.5 μg/ml puromycin for 48 h. Clonal populations were selected using cloning rings and limiting dilution techniques.

Luciferase Assay

The plasmids 4xKBS-pGL3 and p120-pCMV were kindly provided by Dr. Juliet M. Daniel (McMaster University) (Kelly *et al*., 2004). To monitor Kaiso-dependent gene expression, MKN28 cells were cultured in 24-well plates and cotransfected with 100 ng of 4xKBS-pGL3, 4 ng of phRL Null, and 100 ng of p120-pCMV, or 100 ng of the empty vector pCMV for 12 h by using GeneJuice (Novagen, Madison, WI) as recommended by the manufacturer's instructions. Transfection efficiency ranged from 10 to 15%. Eighteen hours after transfection, cells were cocultured with *H. pylori* or medium alone. After 48 h, cells were harvested in 100 μl of reporter lysis buffer (Promega, Madison, WI), and luciferase activity was determined in a dual channel luminometer. Results were normalized for transfection efficiency by cotransfection with the *Renilla* luciferase plasmid (phRL Null).

Transient Transfection of siRNA

MKN28 cells (1.5×10^5) in 12-well plates were transiently transfected using DharmaFECT 2 transfection reagent (Dharmacon RNA Technologies) according to the manufacturer's instructions. Briefly, transfection reagent (1.0 μl/well) was mixed with siRNA oligos (2.5 μl of 20 μm solution/well) in 100 μl of Opti-MEM (Invitrogen). Cells were incubated with the transfection mixture for 24 h, fresh medium was added, and bacterial cocultures were performed 24 h later.

Real-Time Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

MKN28 gastric epithelial cells were grown to confluence and then cocultured with *H. pylori* or medium alone for 2, 4, 8, or 12 h. RNA was prepared from cocultures using TRIzol reagent (Invitrogen) following the manufacturer's instructions. Reverse transcriptase-PCR was performed using TaqMan reverse transcription reagents (Applied Biosystems, Foster City, CA), which was followed by real-time quantitative PCR using the TaqMan gene expression assay and a 7300 real-time PCR system (Applied Biosystems). *mmp-7*, *p120*, and *Kaiso* cDNA were quantified using the human-specific *mmp-7* TaqMan gene expression primer set (Hs00159163_m1), *CTNND1* TaqMan gene expression primer set (Hs00609741_m1), and *ZBTB33* TaqMan gene

expression primer set (Hs00406811_m1), respectively, and expression levels were normalized to levels of 18S rRNA.

Immunofluorescence

Gastric cells were cultured in glass chamber slides and subsequently cocultured with *H. pylori* or medium alone. Six hours after infection, cells were washed twice with Dulbecco's phosphate-buffered saline (DPBS) containing calcium chloride, and then they were fixed in 4.7% paraformaldehyde in DPBS for 15 min at room temperature. Cells were then subjected to antigen retrieval by immersion in deionized water (diH_2O), followed by immersion in citrate buffer, pH 6.0, 8 mM citric acid, and 2 mM sodium citrate tribasic dihydrate) and heating in a 1200-W microwave for 15 min at 20% power. After cooling for 30 min at room temperature, cells were rinsed in diH_2O, incubated with DPBS containing 3% bovine serum albumin (BSA) (Sigma-Aldrich, St. Louis, MO) and 0.1% Triton X-100 for 20 min, followed by incubation in 3% BSA for 1 h at room temperature. Slides were immunostained with mouse monoclonal anti-p120 antibody (pp120; BD Biosciences, San Jose, CA), rabbit anti-p120 antibody (F1αSH), mouse monoclonal anti-Kaiso antibody (6F/6F8; BD Biosciences), mouse monoclonal anti-Kaiso antibody (11D), rabbit polyclonal anti-Kaiso antibody, or rabbit anti-*H. pylori* antibody (Dako North America, Carpinteria, CA) at a concentration of 1:100 overnight at 4°C. Washed slides were incubated with goat anti-mouse AlexaFluor 488-conjugated antibody (Invitrogen), goat anti-mouse AlexaFluor 546-conjugated antibody (Invitrogen), or goat anti-rabbit AlexaFluor 488-conjugated antibody (Invitrogen) at a concentration of 1:100 for 2 h at room temperature. Washed slides were then incubated with TOTO-3 dimeric cyanine nucleic acid dye at a concentration of 1:100 for 20 min at room temperature (Invitrogen). Slides were mounted using ProLong Gold antifade reagent (Invitrogen). Imaging was performed on an LSM 510 confocal microscope (Carl Zeiss, Thornwood, NY) by using a 63×/1.40 Pan-APOCHROMAT oil objective at room temperature, and acquisition was performed with the manufacturer's proprietary software. All three-dimensional reconstructions and fluorescence profile analyses were performed using LSM Image Examiner 3.2 software (Carl Zeiss).

Chromatin Immunoprecipitation

Chromatin immunoprecipitation experiments were performed as described previously (Hearnes *et al*., 2005). Briefly, MKN28 cells were grown to confluence in 10 cm dishes, and then incubated with 1.6% formaldehyde (Sigma-Aldrich) in PBS for 10 min at room temperature. The cross-linking reaction was stopped by adding glycine to a final concentration of 0.14 M. Cells were washed twice with PBS and harvested in 250 μl of chromatin immunoprecipitation (ChIP) radioimmunoprecipitation assay (RIPA) buffer (50 mM Tris, pH 8.0, 150 mM NaCl, 1% NP-40, 0.5% deoxycholate, 0.1% SDS, and 5 mM EDTA) containing protease inhibitor cocktail (P2714; Sigma-Aldrich). Cells were subjected to sonication consisting of eight 10-s pulses at 50% amplitude and centrifuged at 14,000 × *g* at 4°C for 15 min. For each immunoprecipitation, supernatant containing 1 mg of total protein as quantified by the Bradford assay (Pierce Chemical, Rockford, IL) was precleared by the addition of 10 μg of mouse immunoglobulin G (IgG) conjugated to protein A-Sepharose beads (PAS; Zymogen) with rocking at 4°C for 1 h. PAS beads were removed by centrifugation. Two micrograms of antibody and 30 μl of PAS beads were added for each immunoprecipitation, with rocking at 4°C overnight. PAS beads were concentrated by centrifugation and washed twice with 1 ml of ChIP RIPA buffer, four times with 1 ml of immunoprecipitation (IP) wash buffer (100 mM Tris, pH 8.5, 500 mM LiCl, 1% NP-40, and 1% deoxycholate), followed by two washes with ChIP RIPA buffer. During each wash, samples were rotated for 5 min at 4°C. Two hundred μl of cross-link reversal buffer (125 mM Tris, pH 6.8, 10% β-mercaptoethanol, and 4% SDS) was added directly to PAS beads from each sample and boiled for 30 min. DNA was purified by phenol-chloroform extraction and precipitated with ethanol. DNA pellets were resuspended in 20 μl of PCR-grade water. Five microliters of resuspended DNA was subjected to PCR to amplify the *mmp-7* promoter region (forward primer, TAGAGGCAGTGTTCCCCATT; reverse primer, CCAAATCCTGTGGTTCTCC) as described previously (Spring *et al*., 2005). PCR products were electrophoresed on a 1.5% agarose gel at 100 V for 30 min.

Western Analysis

Cells were lysed in RIPA buffer (50 mM Tris, pH 7.2, 150 mM NaCl, 1% Triton X-100, and 0.1% SDS) containing protease inhibitor cocktail, and protein concentrations were quantified by the Bradford assay. Proteins (30 μg) were separated by SDS-polyacrylamide gel electrophoresis (PAGE) and transferred to polyvinylidene difluoride membranes (PVDF, Pall, Ann Arbor, MI). Protein levels were assessed by Western blotting by using mouse monoclonal anti-p120 antibody (1:1000, pp120), anti-E-cadherin antibody (1:1000, BD Bioscience), anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibody (1:2000; Millipore Bioscience Research Reagents, Temecula, CA) or goat polyclonal anti-actin antibody (1:2000, C-11; Santa Cruz Biotechnology, Santa Cruz, CA). Primary antibodies were detected using goat anti-mouse or donkey anti-goat (1:2500; Santa Cruz Biotechnology) horseradish peroxidase-conjugated secondary antibodies and visualized by Western Lightning Chemiluminescence Reagent Plus (PerkinElmer Life and Analytical Sciences, Boston, MA) according to the manufacturer's instructions on a Chemigenius system (Syngene, Frederick, MD). For analysis of exogenous p120 expression, transfected cells were harvested in lysis buffer (20 mM Tris-HCl, pH 7.5, 1 mM ETDA, 100 mM NaCl, 1% Triton X-100, 0.5% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 1× complete protease inhibitors [Roche Diagnostics, Indianapolis, IN], 1 mM Na_3VO_4, 1 mM sodium molybdate, 20 mM NaF, 10 mM sodium pyrophosphate, and 20 mM β-glycerophosphate), separated by SDS PAGE, and transferred to PVDF membranes. Protein levels were assessed by Western blotting by using anti-p120 antibody (pp120) and anti-GAPDH antibody (Abcam, Cambridge, MA).

Subcellular Fractionation

Fractionation was performed as described by Frey *et al*. (1997). Briefly, cells were lysed in low-detergent buffer (20 mM HEPES, 2 mM EDTA, 2 mM EGTA, 0.5 mg/ml digitonin) containing protease inhibitors, centrifuged for 40 min at 100,000 × *g*, and the soluble fraction was collected. The residual pellet was resuspended in high detergent buffer (10 mM Tris, pH 7.4, 2 mM EDTA, 2 mM EGTA, 1% Triton, 0.2% deoxycholate, and 0.1% SDS) containing protease inhibitors, centrifuged for 5 min at 20,000 × *g*, and the membranous fraction was collected.

Immunoprecipitation

Before collection, MKN28 cells were treated with sodium pervanadate to inhibit endogenous phosphatase activity as described previously (Mariner *et al*., 2004). Cells were then lysed in IP lysis buffer (50 mM Tris, pH 7.2, 150 mM NaCl, 1% Triton X-100, 0.1% SDS, and 0.2% sodium deoxycholate) containing protease inhibitor cocktail, phosphatase inhibitor cocktails 1 and 2 (Sigma-Aldrich), and 150 mM vanadate/300 mM H_2O_2; harvested; and protein concentrations were quantified by the Bradford assay. One milligram of total protein was isolated from each sample, and total volumes were equilibrated with IP lysis buffer. Twenty microliters of mouse IgG (Sigma-Aldrich) was added, followed by 30 min of incubation at 4°C with rolling. Then, 20 μl of protein A/G PLUS-agarose immunoprecipitation reagent (Santa Cruz Biotechnology) was added, followed by 30 min of incubation at 4°C with rolling. Samples were centrifuged at 4°C for 10 min at 10,000 × *g*. The supernatants were then isolated and incubated overnight with 1 μg of the appropriate IP antibody (pp120; BD Biosciences; or c-myc, 9E10; Santa Cruz Biotechnology) at 4°C with rolling. Thirty-five microliters of protein A/G PLUS-agarose immunoprecipitation reagent was added to each sample, followed by 1 h of incubation at 4°C with rolling. Samples were centrifuged at 10,000 × *g* at 4°C for 2 min, and the supernatant was discarded. The remaining protein A/G reagent was washed six times with 500 μl of cold temperature PBS containing 150 mM vanadate/300 mM H_2O_2. After the last wash, 75 μl of Laemmli buffer was added to each sample and boiled for 5 min. The samples were centrifuged at room temperature at 10,000 × *g* for 5 min, and the supernatants were analyzed by Western blot analysis. Immunoblotting was performed using a mouse monoclonal anti-phospho-tyrosine antibody (1:200, PY99, Santa Cruz Biotechnology) and anti-phospho-p120 antibodies (1:1000; pY96, pY228, pY291; BD Biosciences).

Quantitative Culture of Adherent Bacteria

H. pylori:MKN28 cell cocultures were washed after 24 h with PBS, pH 7.6, × 2 to remove nonadherent bacteria, and total cell extracts were harvested as described previously (Crawford *et al*., 2003). Serial 10-fold dilutions of 1-ml aliquots of cell extracts were cultured on 5% sheep blood agar plates, and incubated for 3–5 d under microaerobic conditions before *H. pylori* colonies were counted. Results are expressed as colony forming units (cfu) per milliliter.

Statistical Analysis

Student's *t* test was used to evaluate the data, and significance was defined as $p < 0.05$. Fluorescence intensity profiles were generated by creating Lowess spline curves of the XY data output from LSM image examiner software (Carl Zeiss).

RESULTS

H. pylori-mediated mmp-7 Expression Is Transcriptionally Regulated and Is Dependent on a Functional cag Pathogenicity Island

We and others demonstrated previously that MMP-7 protein levels are increased in gastric cells after coculture with *H. pylori* (Crawford *et al*., 2003; Wroblewski *et al*., 2003) and that this is dependent on specific genes within the *cag* island (e.g., *cagE* but not *cagA*) (Crawford *et al*., 2003). *cagE* encodes a homologue of VirB4, a protein involved in toxin export in *Bordetella pertussis*. Based on homology, CagE is postulated to function as a NTPase, which is required for energy-

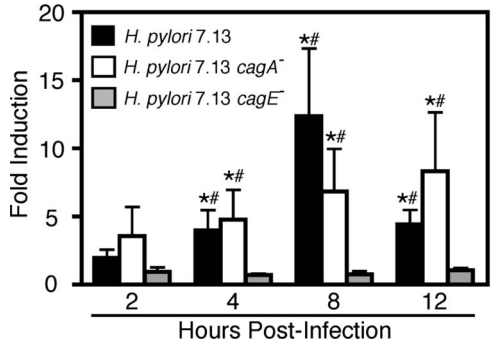

Figure 1. *H. pylori* infection of MKN28 cells results in an increase in *mmp-7* steady-state mRNA levels in a *cagE*-dependent manner. MKN28 cells were cocultured with the *H. pylori* cag^+ strain 7.13 (MOI = 100), the 7.13 $cagA^-$ or $cagE^-$ isogenic mutants, or medium alone. At defined time points, RNA was extracted, subjected to reverse transcription, and analyzed in triplicate by real-time PCR. Data represent -fold induction of *mmp-7* mRNA in cells cocultured with *H. pylori* versus medium alone from experiments performed on at least four occasions. Error bars, SEM. *$p < 0.05$ versus medium alone. #$p < 0.05$ versus $cagE^-$.

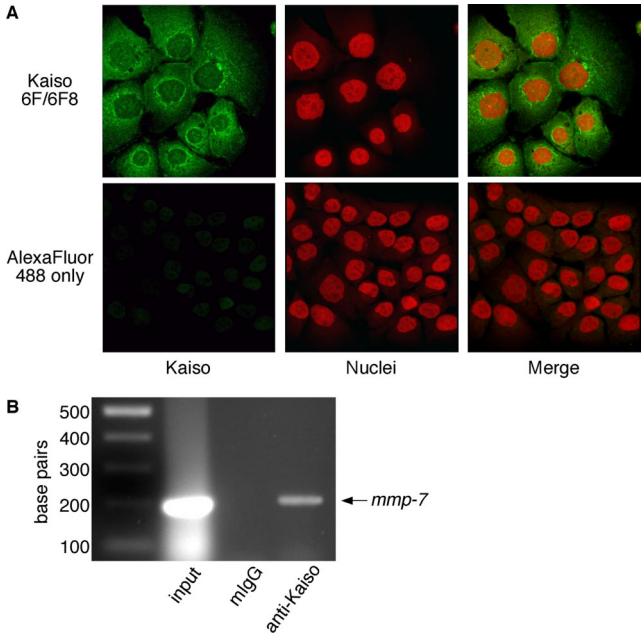

Figure 2. MKN28 cells express the transcriptional repressor, Kaiso, which binds the promoter region of *mmp-7*. (A) MKN28 cells were fixed and incubated with a monoclonal anti-Kaiso antibody (6F/6F8) or PBS alone, followed by incubation with an anti-mouse AlexaFluor-488 antibody and TOTO-3 nucleic acid dye. Cells were visualized by laser scanning immunofluorescent microscopy and are represented as projection images of a Z-stack. Kaiso, green; nuclei, red; 630× magnification. (B) MKN28 cells were subjected to chromatin immunoprecipitation analysis by using a monoclonal anti-Kaiso antibody (6F/6F8), mouse IgG (negative control), or input DNA alone (positive control) followed by PCR amplification by using human *mmp-7* promoter-specific primers. Experiments were performed on at least two occasions. Products were visualized by agarose gel electrophoresis and a representative gel is shown.

dependent delivery of CagA and other *cag* island substrates from the bacterial cytoplasm directly into host cells (Backert and Selbach, 2008). Inactivation of *cagE*, therefore, results in a nonfunctional type IV *cag* secretion system. To determine whether increased MMP-7 protein expression was transcriptionally mediated, MKN28 human gastric epithelial cells were cocultured with the *H. pylori* wild-type cag^+ strain 7.13, a 7.13 $cagA^-$ mutant, a 7.13 $cagE^-$ mutant, or medium alone. At defined time points, steady-state *mmp-7* mRNA levels were assessed by real-time RT-PCR. Levels of *mmp-7* mRNA began to increase 4 h after infection with the wild-type strain 7.13 and peaked by 8 h at levels ~12-fold above control (Figure 1). Inactivation of *cagA* had no significant effect on *H. pylori*-mediated *mmp*-7 induction (Figure 1), confirming our previous results for MMP-7 protein expression (Crawford *et al*., 2003). However, inactivation of *cagE* significantly attenuated *mmp-7* expression (Figure 1), indicating that *H. pylori* strain 7.13 induces *cag*-dependent transcriptional up-regulation of *mmp-7* in human gastric epithelial cells.

Kaiso Binds the mmp-7 Promoter Region in Gastric Epithelial Cells

Recent data have shown that Kaiso mediates transcriptional repression of *mmp-7* in colonic epithelial cells (Spring *et al*., 2005); therefore, we used laser scanning immunofluorescent microscopy to determine whether gastric epithelial cells that produce MMP-7 in response to *H. pylori* (Figure 1) also express Kaiso. As shown in Figure 2A, Kaiso is expressed throughout the cell, including the nuclei, in MKN28 cells. These results were confirmed using additional monoclonal and polyclonal anti-Kaiso antibodies (Supplemental Figure 1).

The human *mmp-7* promoter contains two conserved copies of the consensus Kaiso site, one of which overlaps an ETS transcription factor consensus site and is in proximity to activator protein-1 and TCF/Lef transcription factor binding sites (Spring *et al*., 2005). Kaiso has been shown previously to bind this region of the *mmp-7* promoter in at least three human epithelial cell lines by ChIP assay (Spring *et al*., 2005). Although the immunofluorescence data in Figure 2A indicate that Kaiso is present in the requisite subcellular locale to function as a transcriptional repressor, it is not known whether Kaiso binds to the *mmp-7* promoter in MKN28 cells. Therefore, primers specific for this region of the *mmp-7* promoter were used for ChIP to confirm that Kaiso can bind and regulate *mmp-7* in gastric epithelial cells. *mmp-7* promoter fragments were specifically detected in input fractions and Kaiso immunocomplexes, but not in isotype control immunocomplexes (Figure 2B), indicating that Kaiso binds specifically to the promoter region of *mmp-7* in MKN28 cells.

H. pylori Strain 7.13 Alters Subcellular Distribution of p120 and Induces Aberrant Localization of p120 to the Nucleus

Interactions between p120 and Kaiso can coordinately regulate genes implicated in carcinogenesis (Daniel and Reynolds, 1999; Daniel *et al*., 2002; Kelly *et al*., 2004; Kim *et al*., 2004; Park *et al*., 2005; Spring *et al*., 2005), and translocation of p120 to the nucleus relieves Kaiso-mediated transcriptional repression of *mmp-7* (Kelly *et al*., 2004; Park *et al*., 2005; Spring *et al*., 2005). To determine whether *H. pylori* infection alters total p120 levels, MKN28 cells were cocultured with *H. pylori* strain 7.13 (MOI = 100) or medium alone and subjected to Western blot analysis (Figure 3A). Densitometric analysis of multiple immunoblot experiments indicated that total p120 levels were not significantly altered after infection compared with uninfected controls (data not shown). Similarly, mRNA levels of p120, as measured by real-time RT-PCR, remained unchanged by infection with *H. pylori* (Supplemental Figure 2A).

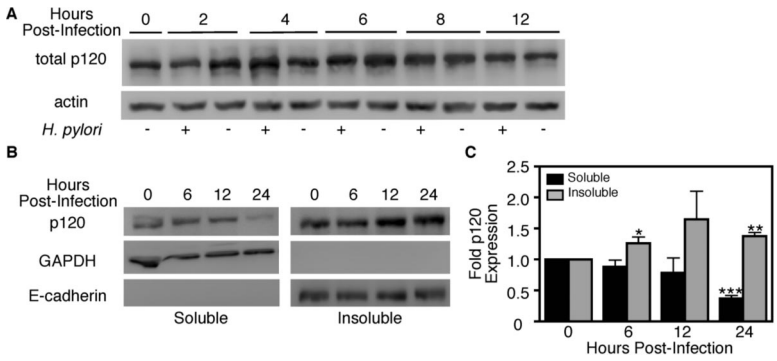

Figure 3. *H. pylori* alters subcellular localization of p120 (A) MKN28 cells were cocultured with *H. pylori* strain 7.13 (MOI = 100) or medium alone. At defined time points, total protein was extracted and analyzed by Western blot by using a monoclonal anti-p120 antibody (pp120). Experiments were performed on at least three occasions. A representative blot is shown. Anti-actin blots served as normalization controls for MKN28 viability under different experimental conditions. (B) MKN28 cells were cocultured with *H. pylori* strain 7.13 (MOI = 100). At defined time points, total protein was extracted, subjected to subcellular fractionation, and analyzed by Western blot by using an anti-p120 antibody. Representative blots are shown. Anti-GAPDH and anti-E-cadherin antibodies served as normalization controls for purification of soluble and insoluble subcellular fractions, respectively. (C) Densitometric analysis of multiple Western blot repetitions performed on at least three occasions. Graph represents fold p120 expression in infected versus uninfected cells. Error bars, SEM. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ versus time 0.

We next sought to determine whether *H. pylori* may induce alterations in subcellular localization of p120. MKN28 cells were cocultured with *H. pylori* strain 7.13, and, at defined time points, total cell lysates were fractionated into a soluble fraction containing cytosolic components and an insoluble fraction containing nuclear and membranous components. Immunoblot analysis of these fractions revealed that *H. pylori* significantly decreased soluble, and concomitantly increased insoluble levels of p120 over 24 h of infection (Figure 3B). Immunoblot analysis performed on cells cocultured with medium alone showed no alteration in soluble or insoluble p120 levels (Supplemental Figure 2B), indicating that increases in cell confluence over time did not contribute to changes in subcellular p120 levels. These results demonstrate that *H. pylori* alters the distribution, but not the total amount, of the cellular p120 pool.

Having identified alterations in the subcellular localization of p120, we next determined whether *H. pylori* strain 7.13 could induce nuclear translocation of p120 in MKN28 cells. Cells were cocultured with *H. pylori* strain 7.13 or medium alone for 6 h, and p120 localization was assessed by laser scanning immunofluorescent microscopy. Nuclear and perinuclear aggregations of p120 were observed in cells cocultured with *H. pylori* compared with medium alone (Figure 4A). As shown in Figure 4B, p120 specifically colocalized with Kaiso in the nuclei of infected but not uninfected cells, placing these molecules in position to potentially regulate genes implicated in carcinogenesis.

H. pylori Strain 7.13 Alters the Phosphorylation State of p120

Having demonstrated that infection with *H. pylori* strain 7.13 induces nuclear translocation of p120, we next sought to identify the mechanism underlying *H. pylori*-induced aberrant subcellular distribution of p120. Previous studies have suggested that phosphorylation of a number of tyrosine residues within the p120 regulatory domain may play a role in p120 signaling (Mariner *et al*., 2004; Shibata *et al*., 2004; Castano *et al*., 2007; Reynolds, 2007). Therefore, we investigated whether *H. pylori* infection similarly altered levels of p120 tyrosine phosphorylation. MKN28 cells were cocultured with *H. pylori* strain 7.13 and, at defined time points, cells were treated with phosphatase inhibitors, harvested, and subjected to immunoprecipitation by using an anti-p120 antibody. Of interest, immunoblot analysis of p120 immunocomplexes using a general anti-phosphotyrosine antibody revealed that *H. pylori* significantly decreased total tyrosine phosphorylation compared with uninfected controls (Figure 5), suggesting that dephosphorylation of p120 tyrosine residues may contribute to alterations in subcellular localization. To define which specific residues might be dephosphorylated, immunoblot analysis was performed using antiphosphoantibodies targeting three specific p120 residues: Y228, Y291, and Y96. However, levels of phosphorylation at each of these residues were not significantly altered by infection (Figure 5A), indicating that other, as of yet uncharacterized, tyrosine phosphorylation sites represent targets for dephosphorylation by *H. pylori*.

H. pylori Relieves Kaiso-mediated Transcriptional Repression in a cag Pathogenicity Island-dependent Manner

H. pylori induces an increase in transcription of *mmp-7* in MKN28 gastric epithelial cells. Having also shown that Kaiso binds the promoter region of *mmp-7* and that *H. pylori* induces aberrant localization of p120 to the nucleus, we next sought to determine whether inhibition of Kaiso-mediated transcriptional repression was required for *mmp-7* transcriptional up-regulation. We used the pGL3–4xKBS plasmid (4xKBS), which carries four tandem copies of the consensus Kaiso binding site upstream of the *luciferase* reporter gene (Kelly *et al*., 2004). Previous studies suggest that the ability to inhibit Kaiso-mediated transcriptional repression of an artificial promoter such as 4xKBS requires p120 levels greater than those endogenously present within cells (Kelly *et al*., 2004). Therefore, we cotransfected MKN28 cells with 4xKBS and a p120 expression vector before *H. pylori* coculture (Figure 6A). Expression of exogenous p120 did not significantly relieve Kaiso-mediated transcriptional repression in uninfected cells (Supplemental Figure 3). However, coculture with *H. pylori* strain 7.13 resulted in a significant inhibition (~2.5-fold) of Kaiso-mediated repression of luciferase expression compared with medium alone (Figure 6B). Inactivation of *cagE*, but not *cagA*, significantly attenuated the ability of *H. pylori* strain 7.13 to relieve Kaiso-mediated transcriptional repression (Figure 6B). These results demonstrate that *H. pylori* relieves Kaiso-mediated transcriptional repression in a *cagE*-dependent manner and that p120 is likely required for this inhibition.

p120 Is Required for H. pylori-mediated Up-Regulation of mmp-7 Expression

To demonstrate coordinated regulation of *H. pylori*-induced *mmp-7* expression by p120 and Kaiso, siRNA was used to

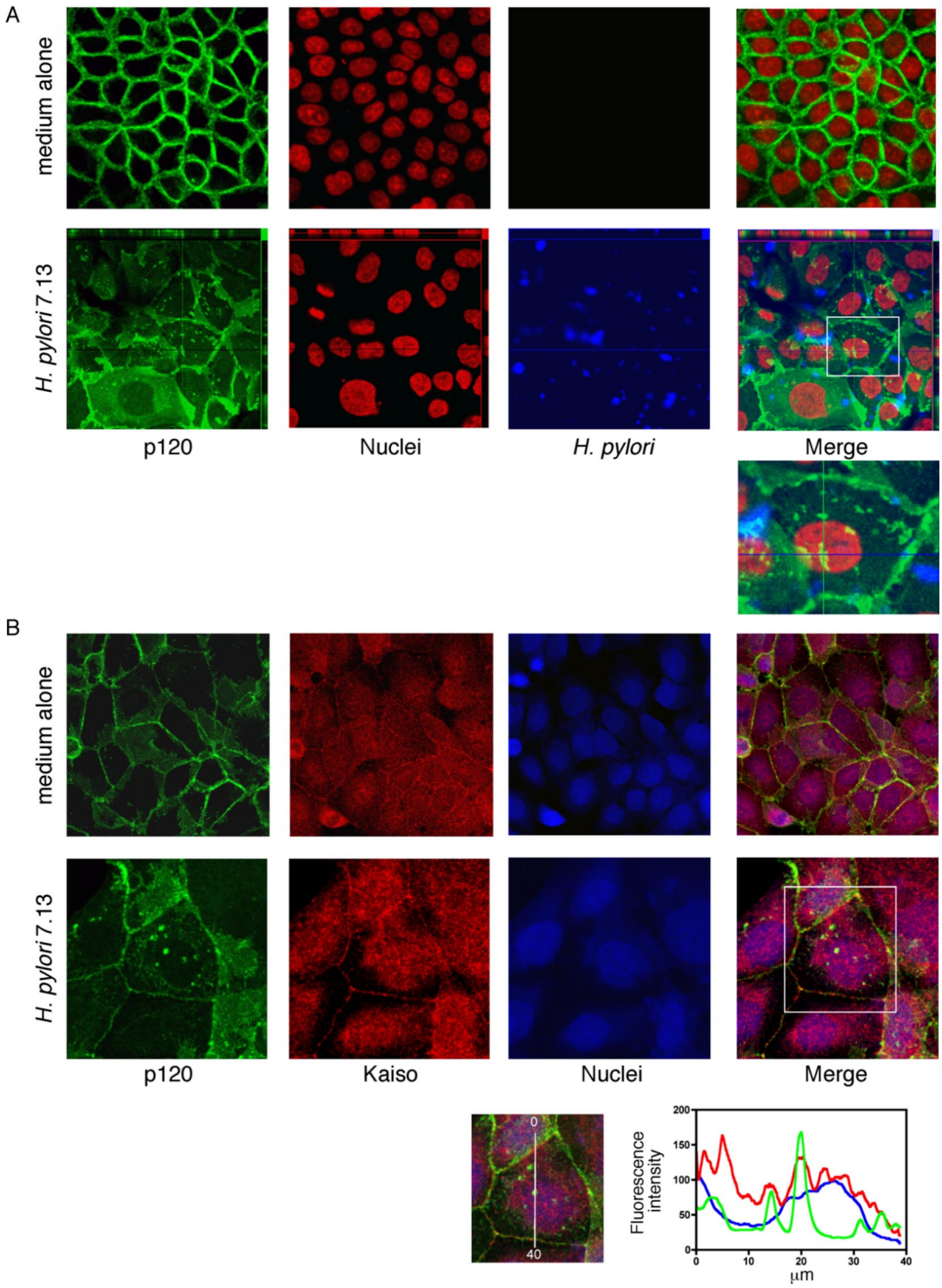

Figure 4. *H. pylori* induces aberrant localization of p120 to the nucleus in MKN28 cells. MKN28 gastric epithelial cells were cocultured with *H. pylori* strain 7.13 or medium alone for 6 h. (A) Cells were fixed and incubated with a monoclonal anti-p120 antibody (pp120) and a polyclonal anti-*H. pylori* antibody, followed by incubation with an anti-mouse AlexaFluor-488 antibody, anti-rabbit AlexaFluor-546 antibody, and TOTO-3 nucleic acid dye. Cells were visualized by laser scanning immunofluorescent microscopy and are represented as projection images of a Z-stack. p120, green; nuclei, red; *H. pylori*, blue; 630× magnification. Localization of p120 in three dimensions is represented by orthogonal views of infected cells. (B) MKN28 cells were cocultured with *H. pylori* strain 7.13, or medium alone, respectively, for 6 h. Cells were fixed and incubated with a rabbit polyclonal anti-p120 antibody (F1αSH) and a monoclonal anti-Kaiso antibody (6F/6F8), followed by incubation with an anti-mouse AlexaFluor-546 antibody, anti-rabbit AlexaFluor-488 antibody, and TOTO-3 nucleic acid dye. Cells were visualized by laser scanning immunofluorescent microscopy and are represented as a single transverse image through the middle of the cell. p120, green; Kaiso, red; nuclei, blue. Uninfected, 630× magnification. Infected, 1260× magnification. Graph indicates fluorescence intensity as measured linearly through the nucleus, demonstrating colocalization of Kaiso and p120 within the nuclei of infected cells.

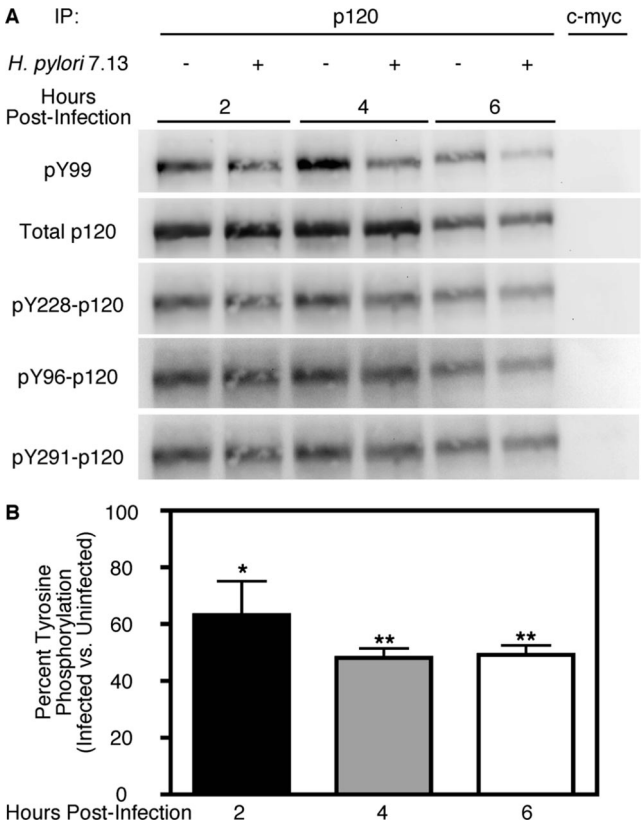

Figure 5. *H. pylori* infection of MKN28 cells results in reduced levels of p120 tyrosine phosphorylation. (A) MKN28 cells were cocultured with *H. pylori* strain 7.13 (MOI = 100) or medium alone. At defined time points, total protein was extracted, subjected to immunoprecipitation with an anti-p120 antibody (pp120), and analyzed by Western blot by using a total anti-phosphotyrosine antibody (pY99) or anti-phosphoantibodies specific to the p120 tyrosine residues 228, 96, or 291. Experiments were performed on at least three occasions. A representative blot is shown. Anti-p120 blots (pp120) served as normalization controls for MKN28 viability under different experimental conditions. As a negative control, 500 μg of total protein from uninfected and infected samples taken 6 h after infection was subjected to immunoprecipitation with an anti-c-myc antibody (9E10). (B) Densitometric analysis of multiple Western blot repetitions performed on at least three occasions. Graph represents percent total tyrosine phosphorylation as determined by Western blotting with pY99 antibody in infected versus uninfected cells. Error bars, SEM. *$p < 0.05$, **$p < 0.001$ versus uninfected cells treated with medium alone.

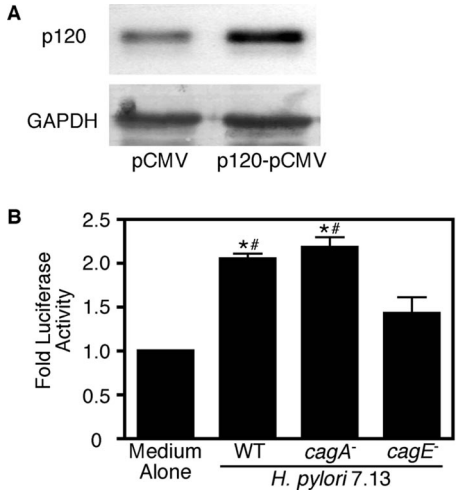

Figure 6. *H. pylori* infection of MKN28 cells mediates a release of Kaiso from its putative binding site on a luciferase reporter plasmid. MKN28 cells were cotransfected with 100 ng of 4xKBS-pGL3, 4 ng of phRL Null, and 100 ng of the empty vector pCMV or 100 ng of p120-pCMV for 12 h. (A) Eighteen hours after transfection, total protein was extracted and analyzed by Western blot by using a monoclonal anti-p120 antibody (pp120). A representative blot is shown. Anti-GAPDH blots served as normalization controls for MKN28 viability under different experimental conditions. (B) Eighteen hours after transfection with p120-pCMV, cells were cocultured with *H. pylori* strain 7.13 or its *cagA*$^-$ or *cagE*$^-$ isogenic mutants. After 48 h, cells were harvested and luciferase activity was determined in a dual-channel luminometer. Data are represented as -fold luciferase activity for experiments performed on at least three occasions. Error bars, SEM. *$p < 0.01$ versus medium alone. #$p < 0.05$ versus *cagE*$^-$.

suppress levels of endogenous p120. MKN28 cells were retrovirally transduced with pRetroSuper vector containing sequences encoding either scrambled siRNA or human-specific p120 siRNA. Clonal populations of transduced cells were isolated and expanded, and a >75% reduction in levels of p120 was achieved (Figure 7A). Because *H. pylori* has been shown previously to bind to components of epithelial junctional complexes, we established that p120 deficiency did not alter bacterial binding to MKN28 cells. As assessed by quantitative culture, *H. pylori* strain 7.13 bound to cells transduced with p120-specific siRNA as avidly as cells transduced with scrambled siRNA, thereby eliminating the possibility that differences in bacterial adherence mediate p120-dependent responses (Figure 7B).

MKN28 cells stably transduced with control or p120-specific siRNA were then cocultured with *H. pylori* strain 7.13 or medium alone. At defined time points, steady-state *mmp-7* mRNA levels were assessed by real-time RT-PCR. As expected, levels of *mmp-7* mRNA were significantly increased by 8 h in control MKN28 cells infected with *H. pylori*, but this increase was attenuated in infected p120-deficient cells (Figure 7C), indicating that p120 is required for *H. pylori* to induce transcription of *mmp-7* in this system. Results were confirmed in two other clonal cell populations transduced with p120-specific siRNA (data not shown).

Suppression of Kaiso in p120-deficient Cells Restores the Ability of H. pylori to Induce mmp-7

To more robustly demonstrate that p120 and Kaiso coordinately regulate *H. pylori*-induced *mmp-7* expression, we transiently transfected control or p120 deficient MKN28 cells with scrambled or Kaiso-specific siRNA. Real-time RT-PCR analysis indicated that Kaiso expression was significantly reduced using Kaiso-specific, but not scrambled, siRNA (Figure 8A). *H. pylori* strain 7.13 was then cocultured with p120-deficient/Kaiso-deficient, p120-deficient/Kaiso-wild-type, or wild-type control MKN28 cells, and *mmp-7* mRNA expression was quantified by real-time RT-PCR. Inhibition of Kaiso in p120-deficient cells restored the ability of *H. pylori* to induce expression of *mmp-7* (Figure 8B), indicating a role for both of these transcriptional elements in *H. pylori*-mediated up-regulation of MMP-7.

H. pylori Induces Aberrant Localization of p120 to the Nucleus in Ex Vivo Gastric Cell Colonies

Our current data demonstrate that *H. pylori* induces increased transcription of *mmp-7* through a p120-dependent mechanism involving inhibition of Kaiso-mediated tran-

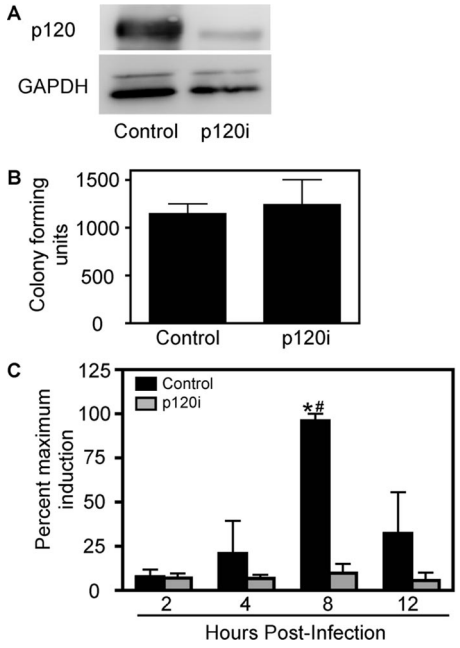

Figure 7. p120 is required for the *H. pylori*-mediated increase in steady-state *mmp-7* mRNA levels. (A) MKN28 cells were retrovirally transduced with either scrambled (control) or human p120-specific siRNA (p120i), and clonal populations were selected. Total protein was extracted from control or p120i cells and analyzed by Western blot using a monoclonal anti-p120 antibody (pp120). GAPDH Western blots served as normalization controls for cell viability under different experimental conditions. (B) Control or p120i cells were incubated with *H. pylori* strain 7.13 (MOI = 100) for 24 h. Cells were washed to remove nonadherent bacteria, harvested, and plated in serial dilutions on blood agar plates. Data represent the number of colony forming units for experiments performed on at least three occasions. (C) Vector control or p120i cells were cocultured with *H. pylori* strain 7.13 (MOI = 100) or medium alone. At defined time points, total RNA was extracted, subjected to reverse transcription, and analyzed in triplicate by real-time PCR. Data are represented as the percentage of maximum induction of *mmp-7* expression in infected versus uninfected cells for experiments performed on at least three occasions. Error bars, SEM. *$p < 0.05$ versus medium alone. #$p < 0.05$ versus p120i cells.

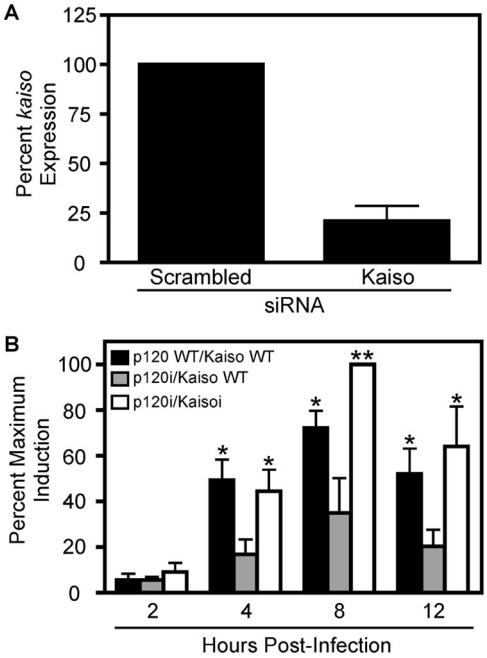

Figure 8. Suppression of Kaiso protein expression in p120i cells restores *H. pylori*-mediated increases in *mmp-7* mRNA levels. Control or p120i cells transiently transfected with scrambled siRNA, and p120i cells transiently transfected with Kaiso-specific siRNA were cocultured with *H. pylori* strain 7.13 (MOI = 100) or medium alone. At defined time points, total RNA was extracted, subjected to reverse transcription, and analyzed in triplicate by real-time PCR. (A) Data are represented as an average percentage of *kaiso* expression in uninfected p120i cells at each time point for experiments performed on at least three occasions. (B) Data are represented as the percent maximum induction of *mmp-7* expression in infected versus uninfected cells for experiments performed on at least three occasions. Error bars, SEM. *$p < 0.05$ and **$p < 0.01$ versus p120i cells.

scriptional repression. We next extended these results using a model of *H. pylori* infection that more closely recapitulates cellular organization in the stomach. Gastric cell colonies were isolated from 8-wk-old male FVB/n mice, cocultured with the mouse-adapted *H. pylori* strain SS1 or medium alone for 6 h, and p120 localization was assessed by laser scanning immunofluorescent microscopy. Mouse-adapted *H. pylori* SS1 was used for these studies because gastric cell colonies were harvested from mice. Consistent with our results in MKN28 cells, nuclear aggregations of p120 were observed to specifically colocalize with Kaiso in cells cocultured with *H. pylori*, but not in cells incubated with medium alone (Figure 9, A and B). These results indicate that, similar to cultured gastric epithelial cells, *H. pylori* can alter p120 localization in a model system that approximates events within colonized gastric mucosa.

DISCUSSION

MMP-7 is a host effector that mediates carcinogenesis and is induced by *H. pylori* within infected human gastric mucosa. Our current experiments identified the p120/Kaiso signaling pathway as a regulator of microbially induced expression of this carcinogenic factor by 1) demonstrating that *H. pylori* can alter the topography of p120 localization within gastric epithelial cells in vitro and in a physiologically relevant ex vivo primary gastric cell culture system, 2) establishing that *H. pylori* can inhibit Kaiso-mediated transcriptional repression of an artificial luciferase promoter, 3) capitalizing on a gastric cell model of p120 deficiency to demonstrate a requirement for p120 in the transcriptional upregulation of *mmp-7*, and 4) combining transient gene silencing techniques with a p120-deficient model system to delineate an interaction between p120 and Kaiso. Collectively, these studies indicate that *H. pylori* coopts p120 as a signaling molecule to relieve Kaiso-mediated repression of *mmp-7*.

p120 is a multidimensional protein that performs several distinct functions within host cells. p120 can regulate E-cadherin, a cell–cell adhesion molecule that functions as a component of the adherens junction of epithelial tissues, and turnover of E-cadherin is regulated by binding of p120 to the cadherin juxtamembrane domain (Reynolds *et al.*, 1989; Reynolds *et al.*, 1992, 1994; Shibamoto *et al.*, 1995; Staddon *et al.*, 1995; Anastasiadis *et al.*, 2000; Thoreson *et al.*, 2000; Anastasiadis and Reynolds, 2001; Ireton *et al.*, 2002; Davis *et al.*, 2003; Reynolds and Carnahan, 2004). Studies have demonstrated that loss of E-cadherin or overexpression of p120 results in mislocalization of p120 to the cytoplasm (Reynolds and Carnahan, 2004), where it induces a range of morpho-

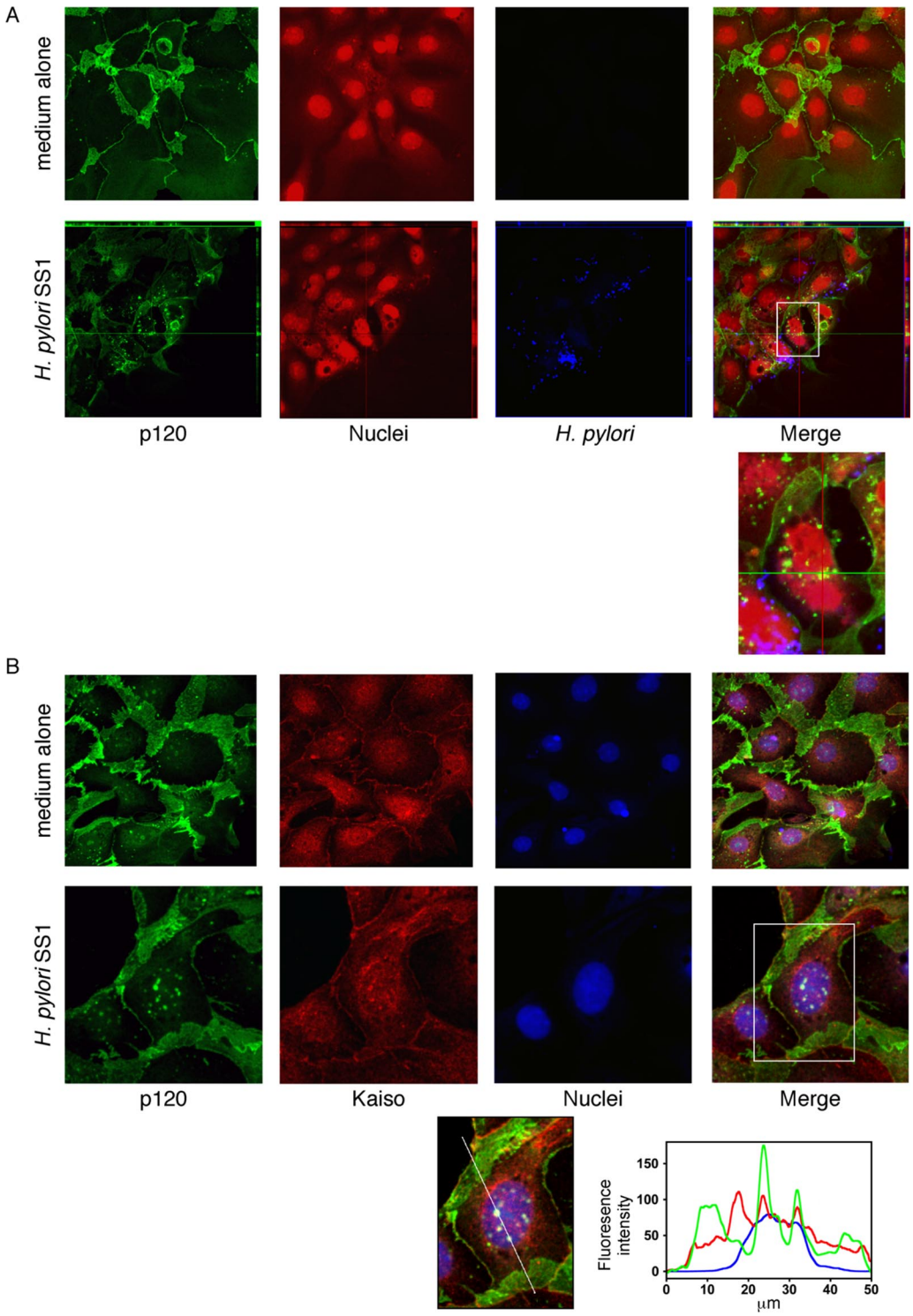

Figure 9. *H. pylori* induces aberrant localization of p120 to the nucleus in ex vivo gastric cell colonies. (A) Primary murine gastric epithelial cell colonies were cocultured with *H. pylori* strain SS1 or medium alone for 6 h. Cells were fixed and incubated with a monoclonal anti-p120 antibody (pp120) and a polyclonal anti-*H. pylori* antibody, followed by incubation with an anti-mouse AlexaFluor-488 antibody, anti-rabbit AlexaFluor-546 antibody, and TOTO-3 nucleic acid dye. Cells were visualized by laser scanning immunofluorescent microscopy and are represented as projection images of a Z-stack. p120, green; *H. pylori*, blue; nuclei, red; 630× magnification. Localization of p120 in 3-dimensions is represented by orthogonal views of infected cells. (B) Primary murine gastric epithelial cell colonies were cocultured with *H. pylori* strain SS1 or medium alone for 6 h. Cells were fixed and incubated with a rabbit polyclonal anti-p120 antibody (F1αSH) and a monoclonal anti-Kaiso antibody (6F/6F8), followed by incubation with an anti-mouse AlexaFluor-546 antibody, anti-rabbit AlexaFluor-488 antibody, and TOTO-3 nucleic acid dye. Cells were visualized by laser scanning immunofluorescent microscopy and are represented as a single transverse image through the middle of the cell. p120, green; Kaiso, red; nuclei, blue. Uninfected, 630× magnification. Infected, 1260× magnification. Graph indicates fluorescence intensity as measured linearly through the nucleus demonstrating colocalization of Kaiso and p120 within the nuclei of infected cells.

logical changes that promote cell motility and metastasis. The effects of p120 seem to involve physical and functional interactions with Ras homolog (Rho) GTPases and their regulators, GTPase-activating proteins and guanine exchange factors (Noren *et al*., 2000; Anastasiadis and Reynolds, 2001; Grosheva *et al*., 2001). Based on our current data, a role for p120 in altering cell morphology and motility induced by *H. pylori* warrants further investigation, and such studies are ongoing in our laboratory.

Nuclear p120 relieves transcriptional repression exerted by Kaiso, which acts as a dual specificity repressor that recognizes both sequence-specific consensus sites (CTGCNA) and methylated CpG nucleotides (Prokhortchouk *et al*., 2001; Daniel *et al*., 2002; Park *et al*., 2005). Because *H. pylori* infection has been associated with gastric cancer and nuclear mislocalization of p120 in human gastric epithelium (Krueger *et al*., 2007), we investigated mislocalization of p120 in conjunction with altered expression of the oncogenic molecule MMP-7. Infection of p120-deficient gastric epithelial cells clearly demonstrated that p120 is required for *H. pylori*-mediated increases in *mmp-7* transcription. Furthermore, silencing Kaiso expression in p120-deficient cells restored the ability of *H. pylori* to induce *mmp-7* transcription, indicating that an interaction between p120 and Kaiso, whether direct or indirect, is also required.

To date, the signals that induce nuclear translocation of p120 remain undefined (Daniel, 2007), and delineation of these pathways is critical for understanding the relevance of p120/Kaiso-mediated transcriptional regulation in the context of carcinogenesis. For example, it will be important to determine which subcellular pool of p120 is responsible for relief of Kaiso-mediated transcriptional repression. Our subcellular fractionation data suggest that p120 from a free cytoplasmic pool translocates to the nucleus. However, work from Weydig *et al*. (2007) demonstrated that *H. pylori* mediates internalization of p120 found in adherens junctions at the plasma membrane, which may then be translocated to the nucleus. p120 is also phosphorylated at a number of sites by Src- and receptor tyrosine kinases (Reynolds, 2007), and multiple ligand–receptor pathways have been implicated in signaling to p120 through phosphorylation, including protein kinase C- and epidermal growth factor receptor-dependent pathways (Mariner *et al*., 2004; Xia *et al*., 2006), both of which are activated by *H. pylori* (Keates *et al*., 2001; Nozawa *et al*., 2004). However, our data demonstrate that *H. pylori* mediates a decrease in total p120 tyrosine phosphorylation, indicating a previously undescribed role for protein tyrosine phosphatases in *H. pylori*-mediated signaling to p120. It remains undefined whether signaling to p120 by *H. pylori* occurs directly or through another cellular intermediary.

Our current results have also provided insight into the augmentation in cancer risk exerted by *cag*$^+$ strains and indicate that oncogenic epithelial responses such as MMP-7 expression may be regulated by different microbial effectors. Previous studies from our laboratory and others have demonstrated that intracellular events with carcinogenic potential, such as β-catenin nuclear translocation, are dependent upon the presence of CagA within host cells, which, in turn, is mediated by a functional *cag* secretion system (Franco *et al*., 2005; Suzuki *et al*., 2005; Murata-Kamiya *et al*., 2007). Our new data indicate that aberrant activation of p120 is dependent on a functional *cag* secretion system, but not CagA per se, suggesting that other substrates translocated by the *cag* island may mediate p120 signaling. One candidate is *H. pylori* peptidoglycan, which is translocated into host cells by the *cag* type IV secretion system and sensed by intracellular Nod1, which then activates NF-κB–dependent responses such as secretion of interleukin-8 and β-defensin 2 (Viala *et al*., 2004; Boughan *et al*., 2006). Another possibility based on a recent investigation (Kwok *et al*., 2007) is that binding of CagL to cell surface $\alpha_5\beta_1$ integrins can alter local membrane dynamics and eventuate in the assembly of focal adhesions that trigger integrin signaling cascades (Backert and Selbach, 2008), which may aberrantly activate p120.

It is of interest that the *H. pylori* strain we used to infect murine gastric colonies (SS1) has been reported to contain a nonfunctional *cag* island. We specifically chose a mouse-adapted *H. pylori* strain for these studies because we were examining murine cells; hence, the choice of strain SS1. However, results from a recent study (Ferrero *et al*., 2008) have suggested that *H. pylori* can activate NF-κB signaling in mouse gastric epithelial cells via a *cag* pathogenicity island-independent pathway, suggesting that signaling pathways in murine cells may be activated by different microbial components than corresponding pathways in human cells. We also did not analyze the functional effects of p120/Kaiso interactions in murine gastric glands, only differences in localization. Differences in model systems may have contributed to these results. In contrast to MKN28 human gastric epithelial cell monolayers, our gastric gland culture model uses tissues isolated from mice that contain not only epithelial cells but also stromal and lamina propria cells. Thus, there are several potential reasons that may explain the ability of *H. pylori* strain SS1 to induce nuclear localization of p120 in murine gastric epithelial cells. Collectively, these data indicate that multiple *H. pylori* disease-related virulence constituents may be required for collaborative functional interactions between Kaiso, p120, and β-catenin in the nucleus to mediate expression of certain Wnt target genes such as *mmp-7*.

Gastric adenocarcinoma is strongly associated with the presence of *H. pylori*, and both microbial and host factors influence the risk for carcinogenesis. Interactions between *H. pylori* and epithelial cells play an important role in the development of gastric injury. p120 is a multifunctional host protein that orchestrates epithelial responses with carcinogenic potential. Our results indicate that p120 is aberrantly activated by *H. pylori* and regulates expression of the carcinogenic effector, *mmp-7*. Molecular delineation of pathways activated by host–microbe interactions will not only improve our understanding of *H. pylori*-induced carcinogenesis but also provide mechanistic insight into other malignancies that arise within the context of inflammatory states (e.g., ulcerative colitis and colon cancer).

ACKNOWLEDGMENTS

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